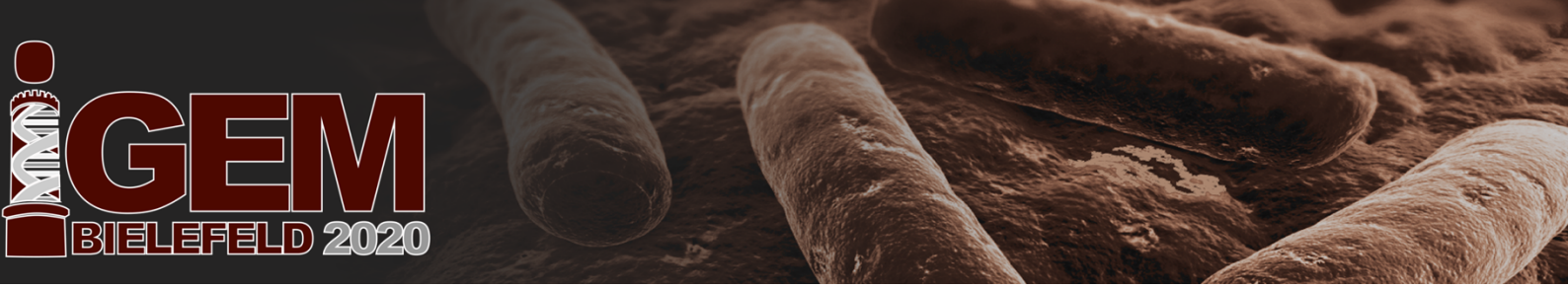

Protocol for DNA Cleanup and Concentration Using the Monarch® PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)

All centrifugation steps should be carried out at 16,000 x g. (~13K RPM in a typical microcentrifuge).

1. Dilute sample with DNA Cleanup Binding Buffer according to the table below. Mix well by pipetting up and down or flicking the tube. Do not vortex. A starting sample volume of 20–100 µl is recommended. For smaller samples, TE can be used to adjust the volume. For diluted samples larger than 800 µl, load a portion of the sample, proceed with Step 2, and then repeat as necessary.

Sample type	Ratio of binding buffer: sample	Example
dsDNA > 2 kb (plasmids, gDNA)	2:1	200 µl:100 µl
dsDNA < 2 kb (some amplicons, fragments)	5:1	500 µl:100 µl
ssDNA > 200 nt*	7:1	700 µl:100 µl

2. Insert column into collection tube and load sample onto column and close the cap. Spin for 1 minute, then discard flow-through.

If using a vacuum manifold* instead of centrifugation, insert the column into the manifold and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

3. Re-insert column into collection tube. Add 200 µl DNA Wash Buffer and spin for 1 minute. Discarding flow-through is optional.
If using a vacuum manifold, add 200 µl of DNA Wash Buffer and switch the vacuum on. Allow the solution to pass through the column, then switch the vacuum source off.

4. Repeat wash (Step 3).

5. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-spin for 1 minute to ensure traces of salt and ethanol are not carried over to next step.
If using a vacuum manifold: Since vacuum set-ups can vary, a 1 minute centrifugation is recommended prior to elution to ensure that no traces of salt or ethanol are carried over to the next step.

6. Add ≥ 6 µl of DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

From: NEB